THE RÔLE OF FERTILIZIN IN THE FERTILIZATION OF EGGS OF THE SEA-URCHIN AND OTHER ANIMALS

ALBERT TYLER

(From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology)

Introduction

The striking phenomenon of the specific agglutination of spermatozoa by a substance obtained from the eggs has been described in a number of species of marine animals (see Lillie, 1919; Just, 1930; Tyler, 1940*a*). Lillie considered this substance, which he called fertilizin, to play a central rôle in the fertilization process, and developed a theory of the mechanism of fertilization based on the ability of fertilizin to combine with the spermatozoön and with some substance within the egg. One of the principal arguments for his views was the evidence that eggs of the sea-urchin which had been deprived of fertilizin lost their capacity for fertilization. In his first experiments (1914) the fertilizin was removed by prolonged washing of the eggs (*Arbacia*), combined in some cases with shaking to remove the jelly layer which he had shown (1913) to be heavily charged with fertilizin. Loeb (1914, 1915) raised the objection that the decrease in fertilizability was due to the aging and death of the eggs during the washing period of one to three days. He showed, on the other hand, that fresh eggs of *Strongylocentrotus purpuratus*, that had been deprived of their jelly layer (which he considered identical with fertilizin) by means of acidified sea water, would still give 100 per cent fertilization. Lillie (1915) repeated the acid sea water experiments with *Arbacia* and found the capacity for fertilization (per cent fertilized) to be much reduced as a result of the treatment. He also noted that some fertilizin could be obtained from the acid-treated eggs although the jelly layer appeared to be completely gone. Later (1921, footnote p. 16), with *Strongylocentrotus*, he found that acid-treated jellyless eggs could still be fertilized although there could not be obtained from these eggs sufficient fertilizin to agglutinate the spermatozoa. He interpreted that result to mean that "an amount of fertilizin insufficient for sperm agglutination is yet adequate for fertilization."

The present experiments resolve these differences as apparently being due to differences in amount of sperm employed for insemination. The

results show that jellyless, fertilizinless eggs can be fertilized but that they must be inseminated with much higher concentrations of sperm than are necessary for untreated eggs. The present, as well as some of the earlier work of the author (1939, 1940), also lends support to Lillie's view that fertilizin is concerned in the fertilization process, and some suggestions are made here as to its rôle. It is further shown that the sperm agglutinating property of fertilizin can be destroyed without altering its ability to combine with the sperm. An interpretation is offered of the temporary nature of the agglutination reaction in the sea-urchin and its more permanent nature in forms like the keyhole limpet. Evidence is also presented that fertilizin is not merely confined to those species of animals whose egg water causes iso-agglutination of sperm, but is more generally distributed and may very likely be universal.

Identity of Fertilizin with the Gelatinous Coat of the Egg

It has been shown (Tyler, 1940*a*) in experiments with the sea-urchin *Strongylocentrotus purpuratus* and the keyhole limpet *Megathura crenulata* that the sperm agglutinin (fertilizin) is located in the jelly layer surrounding the egg. On the rather reasonable assumption that the material of the jelly is a single substance, this means that fertilizin is identical with the jelly. In any event, the evidence showed that fertilizin is a component of the jelly layer and is not secreted by the ripe eggs. Removal of the jelly layer was readily accomplished by means of sea water acidified to between pH 4.5 and 3.5 and also by means of a 1 per cent solution of chymotrypsin in sea water. No fertilizin could be obtained from such jellyless eggs even after prolonged standing. When ripe eggs are allowed to stand in sea water, the jelly slowly goes into solution and the concentration of fertilizin in the solution increases. But this, it was shown, does not increase the total amount of fertilizin that can be obtained from the eggs. In other words, extraction of freshly shed eggs with acid sea water gives just as much fertilizin as that in the acid extract of eggs that had stood for some time in sea water plus that in the supernatant sea water.

Hartmann, Schartau and Wallenfels (1940) support the view that fertilizin is identical with at least a part of the material of the jelly layer. They found in *Arbacia pustulosa* that fertilizin is given off in repeated changes of sea water as long as remains of the jelly layer are present on the eggs. They also removed the jelly layer by means of a sperm extract containing an antifertilizin (see Frank, 1939; Tyler, 1939*a*, 1940*b*; Tyler and O'Melveny, 1941) and obtained no fertilizin from the treated eggs. Further evidence for this view is given by their finding that *Ar-*

bacia sperm agglutinate on the surface of the jelly layer of *Astropecten* eggs but fail to do so if the eggs are first treated with the sperm extract which forms a precipitation membrane on the surface of the jelly.

Additional evidence along this line is contained in some experiments by Evans (1940). He found that Roentgen radiation caused an immediate disappearance of the jelly from around the *Arbacia* egg. Using Janus green as a test for the presence of jelly in egg water, he found that after an irradiation of 59,000 *r* or more it could not be demonstrated in the egg water. He also noted that the agglutinating power of the egg water is greatly reduced after irradiation, and this agrees with Richards and Woodward's (1915) earlier results.

Fertilization after Removal of Fertilizin

The primary question concerning the rôle of fertilizin is whether or not its complete removal in a non-injurious manner renders the eggs non-fertilizable. This question was examined in some experiments with eggs and sperm of the west coast sea-urchin *Strongylocentrotus purpuratus*. Since, as the evidence shows, fertilizin is identical with, or at least a component of the gelatinous coat of the egg, its removal involves the dissolution of this coat. In *Strongylocentrotus,* the jelly is rapidly dissolved by placing the eggs in sea water acidified to between pH 3.5 and 4.5 (Tyler and Fox, 1940). If the eggs are not allowed to remain too long in the acid sea water, there is no visible sign of injury.

Although the jelly is colorless and transparent, its absence is readily noted by the fact that the eggs can then be brought into contact with one another by their surfaces (Tyler, 1940*b*, Fig. 1, *d*). When eggs of *Strongylocentrotus* are deprived of their jelly coat and washed, no detectable (by agglutination of sperm) amount of fertilizin can be obtained either by allowing them to remain for prolonged periods in sea water or by macerating and extracting them with various solvents (Tyler and Fox, 1940).

Upon insemination the jellyless eggs are capable of fertilization to the extent of 100 per cent, as Loeb (1914, 1915) and Lillie (1921) had reported for eggs of *S. purpuratus*. One typical experiment may be cited. Two 20 cc. samples of a 0.1 per cent suspension of fresh *S. purpuratus* eggs in sea water were taken and one of them acidified to pH 4.0. After 5 minutes both dishes of eggs were given a set of four washings with a total of 100 cc. of sea water, allowing the eggs to settle and 1 cc. of suspension to remain in the dishes between washings. The acid-treated eggs were observed to be deprived of their jelly. The addition of 0.05 cc. of a 1 per cent fresh sperm suspension gave 100 per cent

membrane elevation and cleavage in both the acid-treated and control eggs. Similar results were also obtained when the jelly was removed with chymotrypsin.

It may be concluded from this evidence that fertilizin is not essential for fertilization. However, such a conclusion is only valid if the fertilizin has in fact been completely removed from the treated eggs. That this may not be the case is indicated by other evidence and considerations presented below. But, even if it be assumed for the present that fertilizin is not essential for fertilization, the question may still be raised as to whether or not it is an aid to fertilization.

Fertilizin as an Aid to Fertilization

It is well known that the number of spermatozoa required for fertilization is in general much greater than the number of eggs present in the suspension, and as the number of spermatozoa employed for insemination is decreased, the percentage fertilization decreases. The factors responsible for this fact, that many more than one spermatozoön per egg must in general be present in the suspension in order for fertilization to succeed, have been examined by several investigators (Glaser, 1915; Lillie, 1915; Cohn, 1918; Morgan, 1927, p. 27 et seq.), and will not be discussed in any detail here. The present question is whether or not more spermatozoa are required for fertilization when fertilizin is removed from the eggs. This question was investigated with eggs and sperm of *Strongylocentrotus purpuratus* and the results do, in fact, show a decrease in "fertilizability" (increase in amount of sperm required for fertilization) upon removal of the jelly.

Table I lists the results of nine experiments in which the jelly was removed by means of acidified sea water or chymotrypsin. In all cases the eggs were washed after treatment and the pH brought back to that of normal sea water. In the table, cleavage rather than membrane elevation is taken as an index of fertilization inasmuch as the treated eggs often fail to form or to elevate fertilization membranes but may nevertheless cleave (see Tyler and Scheer, 1937). The amounts of sperm added are for convenience all given on the basis of a 0.01 per cent sperm suspension although actually the larger amounts of sperm were taken from more concentrated suspensions. In the different experiments there are, as the table shows, considerable variations in the amount of sperm required to give the same percentage fertilization of the control eggs. This may be due to variations in the condition of the sperm and eggs, in aging of the sperm at various dilutions, in temperature, etc. For the point in question, however, it suffices to compare simply the jellyless with

the control eggs in each horizontal line. The results show that with the larger amounts of sperm the jellyless eggs give practically the same percentage fertilization as the controls. But with smaller amounts there are considerable differences. Thus, with small amounts of sperm that give between 75 and 100 per cent fertilization in the control eggs, only 0 to

TABLE I

Fertilization of jellyless and normal eggs of *S. purpuratus* inseminated with various amounts of sperm. The egg suspensions contain 200 to 400 eggs in 5 cc. of sea water.

Experiment	Treatment for removal of jelly coat	Amount of 0.01 per cent sperm suspension added	Percentage cleavage	
			Jellyless eggs	Control eggs
		cc.		
1	30 min. in pH 4.5 sea water	0.1	5	95
		0.5	35	98
		2.0	99	99
2	30 min. in pH 4.0 sea water	0.05	0	20
		0.5	15	90
		5.0	95	95
3	5 min. in pH 3.5 sea water	0.2	0	25
		1.0	55	95
		5.0	100	100
4	10 min. in pH 3.7 sea water	0.2	10	85
5	10 min. in pH 3.9 sea water	0.1	20	100
		1.0	100	100
6	30 min. in 1 per cent chymotrypsin in pH 8 sea water	0.05	0.2	90
		1.0	45	95
7	30 min. in 1 per cent chymotrypsin in pH 8 sea water	0.1	0.1	75
		2.5	100	100
8	10 min. in 1 per cent chymotrypsin in pH 6 sea water	0.25	15	100
		2.5	100	100
9	10 min. in 1 per cent chymotrypsin in pH 6 sea water	0.5	45	95
		5.0	95	95

20 per cent is obtained in the jellyless samples. To get the same percentage fertilization as in the controls, the amount of sperm required for the treated eggs is roughly five to ten times greater. While the variations in the results do not permit an exact figure to be given for this ratio, it is clear that the differences are all in the same direction in each experiment and are well outside the limits of error. It should

also be noted here that, since sufficient sperm gives as much fertilization in the treated eggs as in the controls, there is no particular injurious action of the treatment involved.

Fertilizin as a Barrier to Fertilization

It appears then that the presence of fertilizin on the eggs is an aid to fertilization in that smaller amounts of sperm are required than in its absence. It might be supposed, then, that restoration of the fertilizin would eliminate the difference and that addition of fertilizin to normal eggs would lower the amount of sperm required for fertilization. Unfortunately, no way is as yet known by which the fertilizin can be restored in its normal state; that is, in the form of a gelatinous coat around the egg. When the jelly is dissolved in acidified sea water it does not go back on to the eggs upon neutralization of the suspension but remains in solution. One might, however, enquire whether or not the presence of fertilizin in solution in the egg suspension increases the fertilizing power of the sperm. This was examined with both jellyless and normal eggs, and it was found that, instead of increasing the fertilizing power of the sperm, the presence of fertilizin in solution had the opposite effect. In one experiment the fertilizin was restored in its original amount (but in solution) and in roughly ten times that amount to suspensions of naked eggs. Various amounts of sperm were used for insemination. The lowest quantity of sperm that gave 100 per cent fertilization in the jellyless controls gave only 15 per cent in the sample with original fertilizin content and 0 per cent in that with the ten-fold concentration. In an experiment with normal eggs the smallest amount of sperm that gave 100 per cent fertilization, gave about 35 per cent when an amount of fertilizin roughly equivalent to the content of the eggs was present in solution and 0 per cent when ten times that amount was present.

The presence of fertilizin in solution evidently acts as a barrier rather than an aid to fertilization. This action, it appears, is due to increase in amount of agglutination of sperm that occurs with increase in amount of fertilizin present in the solution. It is not merely the temporary locking up of the sperm in the agglutinates that causes the decrease in fertilizing power, but, as the next section shows, it involves a permanent effect of the fertilizin on the sperm.

Loss of Fertilizing Power as a Result of Agglutination

F. R. Lillie (1913) showed that the agglutination of sea-urchin sperm by egg water (fertilizin) is temporary. On testing the sperm after reversal of agglutination, he found them to have about half the fertiliz-

ing power (fertilized half the percentage of eggs) of the control sperm suspension. He also noted (1919) that after reversal of agglutination the sperm cannot be re-agglutinated. I have confirmed these findings with *S. purpuratus* and have obtained a much greater reduction in fertilizing power of the sperm.

In twelve experiments that were run, the sperm was agglutinated with sufficiently strong egg water, so that further addition of egg water, after reversal, gave no visible agglutination. The agglutination usually lasted 30 to 40 minutes. Insemination with amounts of sperm that were well above the control minimum for 100 per cent fertilization gave in all tests with the agglutinated and reversed sperm between 0 and 3 per cent fertilization. To obtain the same percentage fertilization with the control as with the treated sperm was found to require between a forty- and a two-hundred-fold reduction in the amount of control sperm used for insemination. The possibility was also examined that the reversed sperm might be more capable of fertilizing jellyless eggs, but the results were negative.

Along with this reduction in fertilizing power of the sperm there is no visible sign of any injurious effect after reversal of agglutination, nor is there any reduction in the activity of the sperm. In fact, the egg water, as is well known, increases the activity of the sperm and as measurements of respiratory rate showed (Tyler, 1939*b*) the increase persists long after the reversal of agglutination. The experiments show, then, that sperm which have been agglutinated are, after spontaneous reversal, incapable of fertilization. The small percentages of fertilization that result when large amounts of treated sperm are used are evidently due to the fact that some spermatozoa in the treated suspensions may escape being agglutinated.

It may be concluded, then, that some change is produced in the spermatozoa, as a result of their reaction with fertilizin, which, although essentially non-injurious, renders them incapable of fertilizing normal eggs. This change might occur during the initial reaction or upon the spontaneous reversal of the agglutination.

The Spontaneous Reversal of Sperm-Agglutination in Sea-Urchins

The temporary nature of the agglutination reaction exhibited by sea-urchin sperm in egg water is an exceptional affair. In the usual serological reactions, the agglutination of various types of cells (blood cells, spermatozoa, bacteria, etc.) by their antisera does not spontaneously reverse, but persists indefinitely. Natural agglutinins, too, such as the

blood group agglutinins in humans, give permanent agglutination which can only be reversed by special treatment. It is of interest, then, not only in connection with fertilization, but in regard to the nature of agglutination reactions in general, to consider the possible causes of the spontaneous reversal.

We shall use as a basis of the present discussion the lattice or framework theory of Heidelberger (1938) and Marrack (1938). This theory postulates that in agglutination as well as precipitation reactions the antigen and antibody are structurally complementary and both are multivalent in regard to their combining groups. Thus one molecule of antigen may combine with more than one molecule of antibody which in turn may combine with more than one molecule of antigen and so build up large aggregates. Where both of the complementary substances are in solution, precipitation results. Where one is present as the surface of the cell, agglutination occurs. The following interpretations may then be suggested for reversal of agglutination in the sea-urchin. (1) The fertilizin molecules plus the combined antifertilizin split off from all of the spermatozoa, leaving neutralized fertilizin in the suspension. (2) They split off at some, rather than all, combining sites in such a way that each (completely neutralized) fertilizin molecule remains attached to not more than one spermatozoön. (3) The fertilizin molecules are split by the action of the sperm leaving univalent fragments combined with the antifertilizin on all the spermatozoa.

All three of these interpretations can account for the failure of reagglutination and the loss of fertilizing capacity on the part of the reversed sperm. Attempts were made to eliminate one or another of these possibilities but the experiments were inconclusive and need not be described here. However, some new findings and further consideration of earlier work lend support to the third interpretation.

It was shown (Tyler and Fox, 1940) that fertilizin of the keyhole limpet is much more resistant than that of the sea-urchin to inactivation by heat and proteolytic enzymes and that this greater stability correlates with the more permanent nature of the agglutination reaction in that form. That the difference is not due to differences in the relative amounts of fertilizin involved is evident by the fact that the reaction is of long duration in the keyhole limpet even when weak fertilizin solutions are employed, whereas it does not in the sea-urchin surpass a maximum of very much shorter duration when the strongest available fertilizin solutions are added. This suggests then that, in the sea-urchin, the combined fertilizin may be broken down fairly rapidly by action of the sperm. It has also been noted that when fertilizin solutions are heated or treated with proteolytic enzymes there is at first a small but definite

increase in activity followed by the gradual inactivation. This suggested the possibility that the fertilizin is first split into smaller but still multivalent molecules. Such behavior is not unique for it has been frequently noted with immune antibodies (see Marrack, 1938; Zinsser, Enders and Fothergill, 1939; Petermann and Pappenheimer, 1941) and the altered agglutinin is termed "agglutinoid." It seemed possible then that, by careful inactivation of fertilizin solutions, univalent fragments might be obtained. The "univalent" fertilizin should be incapable of causing agglutination, but should inhibit subsequent agglutination by untreated fertilizin. It should also be expected to be effective in destroying the fertilizing power of the sperm. As will be shown in the next section, both of these effects have been obtained with heat-treated fertilizin solutions. This, then, lends support to the third interpretation of the spontaneous reversal of agglutination; namely, that the fertilizin molecules are split and the univalent fragments remain attached to the combining groups on the sperm.

"Univalent" Fertilizin

In five experiments concentrated solutions of *S. purpuratus* fertilizin that had been purified by previously described methods (Tyler and Fox, 1940) were heated at 90° to 100° C. just up to the time at which the agglutinating activity had practically disappeared. Sperm was then added to samples (at room temperature) of (*A*) the heated solutions, (*B*) the control solutions, and (*C*) sea water, the relative amounts being such that complete agglutination (no reaction to additional fertilizin after reversal) occurred in the control solution. When unheated fertilizin was added to samples of the sperm in *A*, there was either a very weak reaction or no visible agglutination at all. After reversal of agglutination in *B*, normal eggs were inseminated with various amounts of the sperm suspensions. When amounts of sperm were used that, in the case of the sea water controls, *C*, were near the minimum for 100 per cent fertilization, the *A*-sperm gave 0 to 5 per cent (av. 0.5 per cent) and the *B*-sperm gave 0 to 1 per cent (av. 0.2 per cent) fertilization. A further control was run in those experiments where *A*-sperm showed a weak agglutination reaction upon addition of unheated fertilizin. This was done by diluting the control fertilizin to a concentration giving a similar reaction and adding sperm to the dilute solution at the same time and in the same relative amounts as employed in the other solutions. The fertilizing capacity of the sperm in the diluted fertilizin was found to be only slightly lower than that of the sea water control sperm. An absorption experiment was also performed by the addition of excess

sperm to a sample of the heated fertilizin solution and, after centrifugation, the active agent was found to have disappeared from the supernatant solution.

The results show, then, that the agglutinating property of fertilizin can be destroyed without altering appreciably its capacity to combine with the sperm. The heated fertilizin is usually somewhat weaker than the control in its ability to prevent subsequent agglutination and in its ability to destroy the fertilizing power of the sperm. This most likely means that a small amount of the fertilizin is more completely degraded during the heat treatment. It is clear, however, that by careful heat treatment a modified (non-agglutinating) fertilizin can be produced that differs only slightly, if at all, in its ability to combine with the sperm. Since according to the modern theory a specific agglutinating substance is assumed to be multivalent in respect to its specific combining groups, it is reasonable to consider the non-agglutinating substance in this case univalent.

The formation of univalent fertilizin may be assumed to involve the splitting of the molecule into fragments each of which contains a single combining group or it might involve the splitting off of the combining groups alone. In the latter instance the active agent would be expected to be of small molecular size. Dialysis tests showed, however, that the active agent is incapable of passing through a cellophane membrane. The first assumption appears then to be the more likely one. Other properties of the active agent have not as yet been studied except for a preliminary test of its inactivation by heat. It is inactivated in about one and one-half to three times the time required for destruction of the agglutinating property of the original fertilizin.

Fertilizin in Animals not Exhibiting Iso-Agglutination of Sperm

Lillie (1919) and Just (1930) assumed that eggs of all species of animals possessed fertilizin, although they, themselves, had shown that in many species there is no detectable agglutination of sperm by homologous egg water. They regarded the agglutination reaction simply as an indicator for the presence of fertilizin, but they did not offer any evidence or tests that would demonstrate an analogous substance in the absence of the clumping reaction. The present concept of univalent fertilizin has led to the demonstration of specific sperm-combining substances in species in which the agglutination reaction is lacking. If, in a particular species of animal, the fertilizin obtained in the egg water is univalent, then it should give no agglutination of homologous sperm, but it should destroy their fertilizing capacity.

This point was examined in the starfish *Patiria miniata* and in the gephyrean worm *Urechis caupo*. In the starfish, concentrated egg water causes no agglutination of homologous sperm. In *Urechis* there may occasionally be a weak reaction. Concentrated egg waters were prepared from eggs of these two species by extraction with pH 4 sea water. Sperm was then added to the neturalized homologous and heterologous egg waters as well as to sea water and after a few minutes various amounts were taken for insemination of the homologous eggs. In all cases there was found to be a great reduction in the fertilizing capacity of the sperm treated with homologous egg water, while that treated with heterologous egg water showed approximately the same fertilizing capacity as the sea water controls. A typical experiment may be cited. Concentrated *Patiria* and *Urechis* egg waters were prepared from 10 per cent egg suspensions. One part of a 1 per cent *Patiria* sperm suspension was added to nine parts of (*A*) *Patiria* egg water, (*B*) *Urechis* egg water and (*C*) sea water. The same was done with a one per cent suspension of *Urechis* sperm. Insemination of homologous eggs (approximately 200 eggs in 5 cc. of sea water) with 0.05 cc. of these mixtures gave for *Patiria* no fertilization with *A*, 100 per cent with *B* and 99 per cent with *C*. For *Urechis* the results were 100 per cent with *A* and *C* and 0 per cent with *B*.

These results then lend support to the view of Lillie and Just that fertilizin is of general distribution in animals. When appropriate material is available, the investigations will be extended. For the present it is evident in two species of animals that a specific sperm-combining substance is obtainable from the eggs and, since the substance has no agglutinating action on homologous sperm, it may be termed univalent fertilizin.

Discussion

It has been shown that fertilizin, when present in the form of a gelatinous coat, is an aid to fertilization in the sea-urchin. It would also appear from the experiments that fertilizin is not entirely essential to fertilization. But this assumes that all of the fertilizin is removed upon removal of the jelly. While no detectable fertilizin is obtainable from the jellyless eggs, it is quite conceivable that it may be present in combined form on the surface of the egg. It has been shown (Tyler, 1940*b*) that there is an antifertilizin below the surface of the egg and it would be reasonable to assume that the surface of the egg is composed of a fertilizin-antifertilizin complex. Upon removal of the jelly, this combined fertilizin would remain as a monomolecular layer with free spe-

cific combining groups on its outer surface. In support of this view may be cited the observation of Frank (1939) that jellyless as well as normal sea-urchin eggs can be agglutinated by means of an antifertilizin obtained from the sperm. The possibility may then be admitted that fertilizin is indispensable for fertilization but further evidence along this line would be desirable before any attempt is made to develop a theory of fertilization with it as an essential agent.

In regard to the manner in which fertilizin may act as an aid to fertilization there are several possibilities. In the first place it is clearly not merely the greater volume due to the presence of the jelly that is involved, since the spermatozoön must, in any event, reach the surface of the egg for fertilization to ensue. It is possible that the gradient produced, as the jelly slowly goes into solution, exerts a chemotactic effect on the sperm. There is, however, still no general agreement as to chemotaxis. Hartmann (1940) reports demonstrating such action of fertilizin by means of the Pfeffer capillary method, whereas Cornman (1941) could obtain no positive results with that method.

Another possibility is that the jelly serves as a trap for the sperm. This appears reasonable on the basis of the fact that the spermatozoön reacts with fertilizin in solution. One may suppose that, while most of the fertilizin is in the form of a jelly, some of it is in solution in the interstices; or that even as a gel there are some free combining groups available. The formation of a precipitation membrane on the surface of the jelly by the action of antifertilizin (Tyler, 1940*b*) is more readily explainable on the basis of the latter assumption. Trap action would help to explain how fertilizin (as a jelly) acts as an aid to fertilization, since it would restrict the random movements of the spermatozoa to a small volume and thereby increase the chance of fertilization. However, other and more quantitative experiments are needed before decision can be made as to whether or not it alone can account for greater fertilizability of the normal in comparison with the jellyless eggs.

Another possibility is that some structural property of the jelly causes the sperm to approach so that its long axis is normal to the surface. While observations (*see* Morgan, 1927; Chambers, 1933) indicate that a radial approach is more favorable for fertilization, it has not definitely been shown that oblique approach and contact with the surface results in failure of sperm entry.

The possibility should also be considered that the greater fertilizability of the normal eggs is due to the activating effect of fertilizin on the sperm. But before decision can be made as to the value of this factor, it would be important to know that there is no corresponding decrease in the fertilizable life of the sperm.

In connection with these possibilities, it must be recalled that after the sperm has reacted with fertilizin in solution it is incapable of fertilization and that, probably because of this, the presence of fertilizin in solution in a suspension of eggs acts as a barrier to fertilization. Thus excess sperm is required to take up the fertilizin in solution and leave uncombined sperm available for fertilization. It is evident that in normal fertilization the spermatozoön must reach the surface of the egg before the inhibiting action of the fertilizin surrounding the egg has taken place. If, as suggested above, fertilizin in the form of a jelly has only a few superficial combining groups available, it is quite conceivable that they may serve as the initial trap for the sperm but would not be sufficient to neutralize all of the reacting groups on the sperm before the latter has reached the surface of the egg. The increased activity of the sperm upon reaction with fertilizin would also aid its reaching the surface before the fertilization-inhibiting reaction went to completion. While this seems to be the most likely interpretation, it requires considerably more experimental support. Also, it appears that the information so far available does not warrant a detailed discussion of Lillie's theory of fertilization, nor of the recent views of Hartmann (1940), nor of the development of a new theory of the exact function of fertilizin and other specific substances.

It has been shown in the present work that appropriate treatment of sea-urchin fertilizin converts it into a non-agglutinating agent that is still capable of reacting specifically with the sperm. On the basis of the lattice theory of agglutination reactions this altered fertilizin may, quite legitimately, be designated a univalent substance. It was also shown that the egg waters of certain species of animals that do not contain specific sperm agglutinins nevertheless contain specific sperm-combining substances which may likewise be designated univalent. The absence of the agglutination reaction in many species of animals does not, then, mean the lack of fertilizin, if by that term we mean simply a substance that reacts specifically with the sperm.

This concept may also be extended to problems in general immunology. It is well known that certain animals, such as the rabbit and the horse, readily produce upon immunization, specific agglutinins and precipitins. Others, such as the mouse and the rat, produce little or none but do form protective or neutralizing antibodies. It may be suggested, then, that the antibodies produced in the latter species are principally or entirely of the univalent type. This possibility can be readily tested experimentally;—cells treated with the univalent antibodies should be rendered incapable of being agglutinated by the specific agglutinating antibodies obtained in the former species.

Summary

1. It has been shown in the sea-urchin that the presence of fertilizin, in the form of the jelly coat of the egg, serves as an aid to fertilization. In solution it acts as a barrier to fertilization.

2. Confirmation is presented of Lillie's finding that sea-urchin sperm cannot be re-agglutinated after reversal of an initial agglutination. It is also shown that the reversed sperm are incapable of fertilization.

3. Appropriate heat treatment converts fertilizin into a substance that does not cause sperm agglutination but still combines with the sperm as shown by the inability of the sperm to be subsequently agglutinated by ordinary fertilizin and by loss of fertilizing power. In accordance with the assumption of multivalency in the lattice theory of agglutination, the modified fertilizin is assumed to be univalent. It is found to be non-dialyzable.

4. In the starfish and in *Urechis* the egg water is shown to contain a specific sperm-combining substance (univalent fertilizin) that is incapable of causing iso-agglutination of sperm.

5. Of various interpretations of the spontaneous reversal of agglutination in the sea-urchin, a splitting of the fertilizin into univalent fragments is considered the most likely.

6. Reasons are presented for holding open the possibility that fertilizin plays an indispensible part in fertilization. Various possible explanations as to the manner in which it serves as an aid to fertilization are discussed and that involving trap action is considered the most likely.

7. It is suggested that some species of animals produce upon immunization only, or principally, univalent antibodies and a method of determining this point is offered.

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